TITLE: GENETIC DIVERSITY OF EXOPOLISSACARIDES PRODUCING BACTERIA ISOLATED FROM THE PHYLLOSPHERE OF NATIVE CACTACEA CAATINGA

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ABSTRACT:

The phyllosphere of native Caatinga plants, including cacti, is composed of microorganisms that produce exopolysaccharides (EPS). The EPS are subject to several studies for industrial application, being used, among others, as thickeners, gelling agents and emulsifiers. Considering the diversity of microorganisms, molecular biology has allowed us to interpret this data more quickly and sensitively. Thus, this study had the objective of analyzing the genetic diversity of EPS - producing bacteria isolated from the cacti phyllosphere of native Caatinga. The tested isolates comprise the culture bank of EPS-producing bacteria, previously tested. The isolates were activated in Nutrient Broth culture medium and kept under constant stirring (120 rpm) for 24 hours at 28 °C. Extraction of the genetic material from 13 bacteria was performed following the phenol-chloroform-isoamyl alcohol protocol (25:24:1). The presence and quality of the DNA was verified by a 1% agarose gel stained with SYBR Safe. The diversity among the bacterial isolates was analyzed by PCR (44 uL Supermix PCR, 3 ul primer BOX-A1R (5'-CTACGGCA AGGCGACGCTGACG-3 (10 µM) and 3 µl DNA for a final volume of 50 µl). The amplification was done with initial denaturation at 94 °C for 7 min, followed by 35 amplification cycles: denaturation 94 °C for 1 min, annealing at 53 °C for 1 min, extension at 65 °C for 8 min and an extension final at 65 °C for 15 min. Amplification products wer transferred to 2% agarose gel for electrophoresis with a constant voltage (50V) for 300 min, were stained with SYBR safe and photographed. The banding patterns on the gel were converted into a binary matrix, with 1 for the presence and 0 for the absence of bands of a particular size. The similarity matrix was calculated using Jaccard's coeficiente and the dendrogram was constructed with UPGMA algorithm in the software FreeTree. The diversity of EPS-producing bacteria revealed 9 distinct banding pattern amon the 13 bacterial isolates studied. Only 2 groups, one with two isolates and another with 4, showed 100% similarity. The latter was composed of isolates PH15.1, PH16.1, T7.1 and M7.1, which were isolated from 3 different species of cactaceae. The data presented show that the cactus phyllosophere is composed of a genetically diverse group of EPS-producing bacteria, which may contribute to the adaptive success of these families to arid or semi-arid conditions. These isolates are being sequenced for species determination.

Keywords: BOX-A1R, microorganisms, PCR, semiarid

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